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Review

Fibroblast-macrophage reciprocal interactions in health, fibrosis, and cancer

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SUMMARY

Fibroblasts and macrophages are present in all tissues, and mounting evidence supports that these cells engage in direct communication to influence the overall tissue microenvironment and affect disease outcomes. Here, we review the current understanding of the molecular mechanisms that underlie fibroblast-macrophage interactions in health, fibrosis, and cancer. We present an integrated view of fibroblast-macrophage interactions that is centered on the CSF1-CSF1R axis and discuss how additional molecular programs linking these cell types can underpin disease onset, progression, and resolution. These programs may be tissue and context dependent, affected also by macrophage and fibroblast origin and state, as seen most clearly in cancer. Continued efforts to understand these cells and the means by which they interact may provide therapeutic approaches for the treatment of fibrosis and cancer.

INTRODUCTION

"…macrophages and fibroblasts are closely related forms," noted Alexis Carrel and Albert Ebeling in "The Fundamental Properties of the Fibroblast and the Macrophage. II. The Macrophage," the second in their series of four monographs published in the *Journal of Experimental Medicine* from 1926 to 1928 (Carrel and Ebeling, 1926a, 1926b, 1928b, 1928a). These manuscripts explored the morphology, *in vitro* characteristics, modes of locomotion, and other "essential properties" of fibroblasts and macrophages derived from normal human tissues or tumors.

Some of these findings belonged to their time and others proved incorrect. Yet, nearly 100 years on, many conclusions from these studies still resonate. For instance, they noted that fibroblasts do not require a defined growth factor for *in vitro* cultivation, whereas macrophages do (later discovered to be colony-stimulating factor 1 [CSF1]; Carrel and Ebeling, 1926a); that macrophage and monocytes become identical in appearance when cultured in the same conditions (Carrel and Ebeling, 1926b); and that activated fibroblasts liquify fibrin, a component of wounds (Carrel and Ebeling, 1928b, 1928a). Reading these papers also offers the colorful prose required to describe the beauty and complexity of biology before more sophisticated instrumentation could paint these pictures. "A delicate silk veil when blown by the wind" is a poetic way to describe the cell membrane of locomoting macrophages (Carrel and Ebeling, 1926b). Most salient, though, is an observation derived upon the removal and subsequent culture of tumor cells implanted subcutaneously into a rat: "It was obvious that the pure culture of sarcomatous fibroblasts… had attracted macrophages…It may be supposed that the sarcomatous fibroblasts are supplied by the macrophages, directly or indirectly, with certain substances which are growth promoting" (Carrel and Ebeling, 1928b).

We now know that fibroblasts are a predominantly mesenchymal cell lineage that is responsible for defining the topology of tissues through the deposition and remodeling of extracellular matrix proteins (ECMs), such as collagens. Macrophages are cells first identified as phagocytes and components of the "defense force" of natural immunity, as noted by Metchnikoff (Metchnikoff, 1921). In addition to their primary roles, or at least the functions initially ascribed to them, we have learned that both of these cell types perform a suite of functions geared toward achieving tissue homeostasis (Buechler and Turley, 2018; Wynn et al., 2013) through direct interactions, as we will focus here, and through signals derived from other cell types in their microenvironment. The field also appreciates that macrophages can originate during embryogenesis as well as from hematopoietically derived monocytes (reviewed in Guilliams et al., 2020; Lavin et al., 2015). Indeed, fibroblasts and macrophages are found across all organs, often in close proximity (Uderhardt et al., 2019), and may regulate each other as a two-cell circuit (Zhou et al., 2018).

Carell and Ebeling composed their elegant monographs because they felt new technological advances such as the isolation of cells in pure cultures and measurements of cell growth afforded new understandings into the fundamental properties of fibroblasts and macrophages (Carrel and Ebeling, 1926a). The field of immunology is now able to understand the cellular composition of healthy and diseased tissues in heretofore unprecedented dimensionalities due to single-cell technologies. Novel genetic tools are also poised to dissect the interactions of fibroblasts and macrophages with certainty and precision that was previously unimaginable. So, we feel that now is an exciting time to review the current understanding of fibroblast-macrophage bi-directional interactions. To do so, we focus on the molecular underpinnings of how these cells interact in the steady state, during fibrosis, and within the tumor microenvironment (TME). Where possible, we discuss evidence that utilized state-of-the-art *in vivo* targeting of cells and *ex vivo* analyses, while acknowledging historical references. We then attempt to form a conceptual

synthesis that examines how these cells interact in resting and the inflamed conditions of fibrosis and cancer.

FIBROBLAST-MACROPHAGE INTERACTIONS IN HEALTHY TISSUES

Our understanding of macrophage biology in steady-state tissues has progressed immensely since the monographs produced by Carrel and Ebeling. Along the way, fibroblasts have been instrumental in achieving a clearer understanding of macrophages. In the 1960s, it was recognized that tissue homogenates (Sheridan and Stanley, 1971), serum (Robinson et al., 1967), and a factor in human urine (Robinson et al., 1969) could sustain macrophage progenitor colonies *in vitro*. By 1975, von Furth and colleagues utilized conditioned media derived from cultured fibroblasts to grow macrophages *in vitro* (Goud et al., 1975). The essential growth factor for these experiments, CSF1, was purified from a fibroblast cell line, L cells, in 1976 by E.R. Stanley and colleagues (Stanley and Heard, 1977; Stanley et al., 1978).

An array of experiments and papers over the next three decades in the osteopetrotic (*op/op*) mutant mouse, which lacks most macrophages because of a spontaneous mutation, revealed that the paucity of macrophages is due to loss of CSF1 protein (Wiktor-Jedrzejczak et al., 1990), which can be rescued by administering soluble CSF1 (Cecchini et al., 1994; Wiktor-Jedrzejczak et al., 1990). Ryan et al. found that insertion of a *Csf1* transgene encoding full-length CSF1 under the control of the native *Csf1* promoter and first intron into *op/op* mice restored fibroblast-derived CSF1 and macrophage homeostasis (Ryan et al., 2001). In mice and humans, biologically active CSF1 can be found as secreted forms or be membrane bound (Dai and Stanley, 2003), and this protein can be produced by other cells, such as endothelial cells, in addition to fibroblasts. In all cases, CSF1 signals downstream of the receptor tyrosine kinase CSF1R, which is expressed predominately by cells in the macrophage lineage. Accordingly, mice genetically lacking CSF1R (Dai et al., 2002) or animals treated with an antibody blockade of CSF1R (MacDonald et al., 2010) exhibit a loss of most macrophages, in line with observations in *op/op* mice. Alternative ligands of CSF1R, including IL-34 (Wang et al., 2012), have been discovered, but most macrophages require signaling via the CSF1-CSF1R axis.

CSF1 acts by promoting survival, differentiation, and proliferation of macrophages (Roth and Stanley, 1992). The advent of bulk and single-cell RNA sequencing (scRNA-seq) technologies coupled with a more sophisticated understanding of macrophage gene regulation, including that the transcription factor PU.1 is required for macrophage development (Anderson et al., 1998; DeKoter et al., 1998), revealed that CSF1 can also promote macrophage transcriptional identity. In 2013, Mossadegh-Keller et al. demonstrated using the *Pu.1*-GFP transgenic mouse and scRNA-seq that CSF1 elicits the expression of *Pu.1* and the expression of macrophage-associated genes in hematopoietic stem cells (Mossadegh-Keller et al., 2013). Medzhitov and colleagues proposed a symbiotic relationship between fibroblasts and macrophages in 2018, suggesting a two-cell circuit by which these cells reciprocally interact and sustain themselves in tissues (Zhou et al., 2018). In this framework, which was developed *in silico* and tested *in vitro*, fibroblasts sustain macrophages through provision of CSF1. The Bajenoff group reported a significant diminishment of red pulp macrophages in the spleen and large cavity macrophages in the peritoneum upon loss of *Csf1* in red pulp fibroblasts using the $Wt1^{cre}$;$Csf1^{fl/fl}$ mouse, validating that macrophages can rely on fibroblast-derived *Csf1 in vivo* (Bellomo et al., 2020), though this concept requires testing in other organs.

As evidenced by Carrel and Ebeling, fibroblasts have been of interest to scientists for over a century (Carrel and Ebeling, 1926a). However, fibroblast biology has taken longer to unravel relative to the astounding progress achieved in the macrophage field. While excellent work on these cells has increased our understanding, aspects of the development, maintenance, and heterogeneity of fibroblasts remain unclear. However, frameworks are emerging (Buechler et al., 2021), and it is appreciated that quiescent or universal fibroblasts are functionally distinct from activated fibroblasts (Buckley et al., 2015).

Fibroblasts express the class III protein tyrosine kinase receptor platelet-derived growth factor receptor alpha (PDGFRα). This receptor binds to the mitogens of the PDGF family. The two-cell circuit framework suggests that macrophages provide PDGF ligands required for fibroblast survival. There is complexity associated with this putative relationship *in vivo*. PDGFRα is required for embryonic development because homozygous mutant mice are not viable beyond embryonic day 16 (E16) (Soriano, 1997), so examination of the requirement(s) for PDGFRα in adult fibroblasts requires transgenic animals in which PDGFRα can be temporally manipulated. Tallquist and colleagues found that fibroblasts marked by $Tcf21^{mcrem}$ and $Gli1^{creERT2}$ in the heart were diminished by 50% within a week of tamoxifen-mediated loss of *Pdgfra*, using the $Pdgfra^{flox}$ allele, suggesting a requirement for this receptor for fibroblasts *in vivo* (Ivey et al., 2019). The authors used mutant mice and *in vitro* studies to find that Pdgfrα can signal via PI3K to activate an ATF3-mediated survival program in fibroblasts. Curiously, the animals exhibited no overt phenotype even though the fibroblast compartment did not rebound to baseline levels several months after tamoxifen-dependent loss of *Pdgfrα* in fibroblasts. One interpretation of these results is that subset(s) of fibroblasts may be more reliant upon PDGFRα signaling than others, though this remains to be tested. To this end, it is not clear what ligands, if any, are required by fibroblast subsets for homeostasis, let alone what cell type(s) may provide them *in vivo*.

Fibroblasts and macrophages are found in close association in several tissues in the steady state, as found by Germain and colleagues using *in vivo* imaging (Uderhardt et al., 2019). This association could enable reciprocal growth factor signals to be exchanged by fibroblasts and macrophages. Yet, it was reported that splenic red pulp macrophages do not express PDGF ligands in the steady state, indicating that macrophages may not supply these growth factors to fibroblasts in all cases (Bellomo et al., 2020). Other cell types, including endothelial cells, can be an additional source of PDGFs, so if this factor is essential for fibroblasts, it may be provisioned by other cells in addition to macrophages. Overall, in the steady state, it is clear that fibroblasts can be an essential source of CSF1 for macrophages, and more studies are required to evaluate the role of macrophage-derived ligands

Table 1. Key fibroblast-macrophage interactions in the steady state, fibrosis, and the TME

Fibroblast-derived factor(s) or receptor(s)	Monocyte/macrophage-derived factor(s) or receptor(s)	Condition and model	Key functional outcomes	References
Steady state				
CSF1	CSF1R	*Wt1*^{cre};*Csf1*$^{fl/fl}$	Diminishment of red pulp macrophages and large cavity macrophages *in vivo*	Bellomo et al., 2020
PDGFRα	–	*Tcf21*^{mcrem};*Pdgfra*$^{fl/f}$ and *Gli1*^{creERT2};*Pdgfra*$^{fl/fl}$	50% loss of cardiac fibroblasts	Ivey et al., 2019
Fibrosis				
TGFβ Receptor complex	TGFβ1	*Lyzm*^{cre};*Tgfb1*$^{fl/fl}$	Attenuated lung fibrosis in bleomycin model	Young et al., 2016
PDGFRα	PDGF-AA	*Cx3cr1*^{CreERT2}; *Rosa26*$^{LSL.DiptheriaToxinA}$	Attenuated lung fibrosis in bleomycin model	Aran et al., 2019
CSF1	CSF1R	αCSF1R antibody	Diminished radiation-induced lung fibrosis	Meziani et al., 2018
EGFR	AREG	AREG knockout mice	Attenuated lung fibrosis in bleomycin model	Ding et al., 2016
?	?	*Lyve1*$^{cre/GFP}$;*Slcob1*$^{flox/DTR}$ bone marrow chimera	Attenuated lung fibrosis in bleomycin model	Chakarov et al., 2019
?	?	*CD11b*-DTR	Mitigated fibrosis upon early DT treatment or protracted injury upon late DT treatment in livers after carbon tetrachloride treatment	Duffield et al., 2005
PDGFRα	?	PDGFRα$^{+/K}$ (D824V) and PDGFRα$^{+/J}$ (V561D) mutant mice	Constitutive PDGFRα signaling is associated with fibrosis *in vivo*	Olson and Soriano, 2009; Iwayama et al., 2015
Tumor microenvironment				
CSF1	CSF1R	Multiple tumors (e.g., melanoma)	Core molecular program between fibroblasts and macrophages	Neubert et al., 2018
CCL2 (FAP$^+$ CAFs)	CCR2	iCCA; lymphoma; melanoma and breast cancer	Recruit monocytes to become MDSC	Yang et al., 2016; Ren et al., 2012
CXCL14	?	PDAC	Recruit monocytes; fibroblast expansion	Augsten et al., 2009
CXCL16	CXCR6	Breast cancer	Expand CAFs; recruit more myeloid cells	Allaoui et al., 2016
C3a (CD34$^+$ CAFs)	C3aR	Melanoma	Recruit circulating monocytes	Davidson et al., 2020
TGFβR2	TGFβ	Breast cancer	TGFb induces LOX in CAFs to remodel ECM	Maller et al., 2021
IL-1βR	IL-1β	Melanoma	IL-1b induces CAFs to produce IL-8, which then recruit more CXCR2$^+$ cells	Young et al., 2017
PDGFRα	PDGFA/B	Lung cancer; prostate cancer	PDGF/PDGFR signaling correlates with poor prognosis	Vignaud et al., 1994
MMP2	Syndecan-2 (SPP1$^+$ TAMs)	Colon cancer	Predicted preferential interaction between SPP1$^+$ TAMs and CAFs to promote pro-tumor ECM	Zhang et al., 2020
?	Granulin	PDAC	Promote fibroblast activation and tumor growth	Quaranta et al., 2018

In some studies, specific subsets of CAFs or TAMs were indicated (shown here using parentheses). iCCA, intrahepatic cholangiocarcinoma; PDAC, pancreatic ductal adenocarcinoma.

for PDGFRα or other growth factor receptors in fibroblast homeostasis (Table 1).

FIBROBLAST-MACROPHAGE INTERACTIONS DURING FIBROSIS

Fibrotic diseases such as idiopathic pulmonary fibrosis (IPF) and systemic sclerosis are characterized by unrestrained deposition and stiffening of ECM by fibroblasts. Thus, in these disorders, fibroblasts represent effector cells that drive disease, diminished quality of life, and death. The etiology of fibrotic disorders, of which there are many, differs, but it is accepted that macrophages play key roles in the initiation and resolution of fibrosis (Wynn and Barron, 2010). As such, this spectrum of diseases offers an instructive lens to evaluate inflammatory interactions between macrophages and fibroblasts.

As in the steady state, during fibrosis and other forms of inflammation, fibroblasts seem to maintain expression of *Csf1*. The continued provision of this factor provides resident macrophages or newly infiltrated macrophages with a key signal to retain them in a fibrotic lesion. Indeed, activated macrophages increase CSF1 protein production from fibroblasts *in vitro*, although the mechanism by which this occurs is unclear (Meziani et al., 2018). Activated fibroblasts produce the macrophage chemoattractant CCL2, suggesting that these cells can also attract monocytes or macrophages to areas of fibrosis or injury (Meziani et al., 2018; Seki et al., 2007).

The mechanisms by which macrophages may influence fibroblast biology have been addressed to a greater extent in the setting of fibrosis than in steady-state tissues. Duffield et al. showed convincingly that macrophages are key players in fibrosis and set the stage for more mechanistic investigations between these cells. In a seminal study, this group used the CD11b-DTR system to deplete macrophages at the onset of liver fibrosis induced by carbon tetrachloride damage or during the resolution phase of this injury. The findings were striking—the depletion of macrophages at the onset of fibrosis limited the extent of fibrotic injury whereas loss of macrophages during fibrotic resolution prolonged fibrosis (Duffield et al., 2005). Macrophages can affect fibroblasts by direct cell-cell interactions or through indirect signals involving additional cell types and the extracellular milieu.

Mechanistically, as the two-cell circuit posits, one means by which macrophages may participate in the initiation of fibrosis is through the expression of PDGFs. Indeed, increased *Pdgfa/b* expression in macrophages is observed in human IPF (Martinet et al., 1987), activated human alveolar macrophages (Shaw et al., 1991), and mouse models of pulmonary fibrosis (Aran et al., 2019). Our deepening understanding of macrophage transcriptional subsets has raised the possibility that some macrophages, either by innate predisposition or by spatial localization, may be more prone to drive fibrosis than others (Aran et al., 2019; Chakarov et al., 2019; Meziani et al., 2018). In the mouse lung, evidence suggests that interstitial macrophages, which express CX3CR1 and MHCII, are pro-fibrotic. This is supported by elegant work from Bhattacharya and colleagues that revealed that *Pdgfa* expression is increased in *Cx3cr1*-expressing lung macrophages during bleomycin-induced lung fibrosis and that depletion of these cells using the $Cx3cr1^{CreERT2}$;$Rosa26^{LoxStop-Lox(LSL)DiptheriaToxinA}$ model diminished fibrosis. They went further to show that activated macrophages promoted fibroblast migration and proliferation *in vitro* and that this was diminished in the presence of a PDGF-AA blocking antibody (Aran et al., 2019). Other groups have shown that selective depletion of interstitial macrophages using anti-CSF1R antibody or genetically engineered mice can limit lung fibrosis in response to radiation (Meziani et al., 2018) or bleomycin (Chakarov et al., 2019), though the role of PDGFs was not examined in these studies.

Genetic studies focusing on the PDGFRα signaling pathway in fibroblasts have associated stimulation downstream of this receptor and a fibrotic program. Soriano and colleagues generated transgenic mice in which $Rosa26^{creERT2}$ activity promoted the expression of mutant PDGFRα proteins in which point mutations drove constitutive signaling activity. These proteins ($PDGFR\alpha^{+/K}$ (D824V) and $PDGFR\alpha^{+/J}$ (V561D)) were selected as they are the most common PDGFRα mutations in human gastrointestinal stromal tumors. Animals with these mutant PDGF receptors exhibited systemic organ fibrosis 6 months after tamoxifen-induced cre recombination (Olson and Soriano, 2009). Additional studies utilizing $Nestin^{cre}$ to activate the $PDGFR\alpha^{+/K}$ gain-of-function mutant suggested that overexuberant PDGFRα signaling may promote a cell-intrinsic profibrotic program, though the molecular and cellular mechanisms by which this may occur are not clear (Iwayama et al., 2015). Indeed, it may be that PDGFRα signaling promotes fibroblast proliferation, which renders these cells more responsive to molecular cues that can drive a pro-fibrotic phenotype. A hallmark of systemic sclerosis in humans is the presence of autoantibodies to PDGFRα (Baroni et al., 2006), and gain-of-function point mutations in this receptor are associated with some forms of cancer (Heinrich et al., 2003), so further consideration of the role of this pathway in human disease is warranted.

Several lines of evidence suggest that macrophages can be a source of ligands that promote a fibrotic activation program in fibroblasts. Transforming growth factor beta (TGFβ) is considered to be the master regulator of fibrosis (reviewed in Yue et al., 2010). This cytokine exists in three isoforms that are latent complexes. Although it is often assumed that all three latent complexes require processing to become bioactive, the steps leading to release of active cytokine are better understood for TGFβ1 compared with the other isoforms (reviewed in Derynck et al., 2021). In fibroblasts, TGFβ induces expression of a number of factors that are indicative of activated fibroblasts or myofibroblasts, including alpha smooth muscle actin (αSMA), collagen I, cartilage oligomeric matrix protein (*Comp*), periostin, and connective tissue growth factor (CTGF) via SMAD3 activation downstream of the TGFβ receptor 1 subunit/ALK5 (reviewed in Frangogiannis, 2020). TGFβ can be expressed by multiple cell types, including activated macrophages. Genetic deletion of *Tgfb1* from macrophages using the $LyzM^{cre}$ allele greatly diminished lung collagen content and the overall fibrosis score in bleomycin-induced fibrosis (Young et al., 2016).

TGFβ from macrophages, or other sources including fibroblasts themselves, can act as an accessory signal in macrophage-fibroblast interactions in fibrosis. TGFβ and interleukin (IL)-6 can operate in an autocrine loop in rat pancreatic fibroblasts *in vitro* as one growth factor stimulates production of the other (Aoki et al., 2006). Provision of IL-6-IL-6Rα has been shown to promote a pro-fibrotic program in fibroblasts by driving trans-signaling, though the role of TGFβ was not tested here (Khan et al., 2012). Dermal fibroblasts isolated from systemic sclerosis patients receiving tocilizumab, an anti-IL-6Rα blocking antibody, showed significantly diminished protein levels of fibrotic molecules including collagen I, αSMA, and CTGF compared to patients treated with a placebo (Denton et al., 2018). Interestingly, macrophages have been shown to be essential for IL-6 production by fibroblasts *in vitro*, though the mechanism(s) for this is unknown (Ma et al., 2012). TGFβ can synergize with IL17A to promote IL-6 and CCL2 production in human dermal fibroblasts, providing another means to recruit macrophages to fibroblasts (Dufour et al., 2018). IL-6 promotes macrophage activation during fibrosis (Ayaub et al., 2017), and this cytokine also can be produced by macrophages and monocytes in addition to other activated cells.

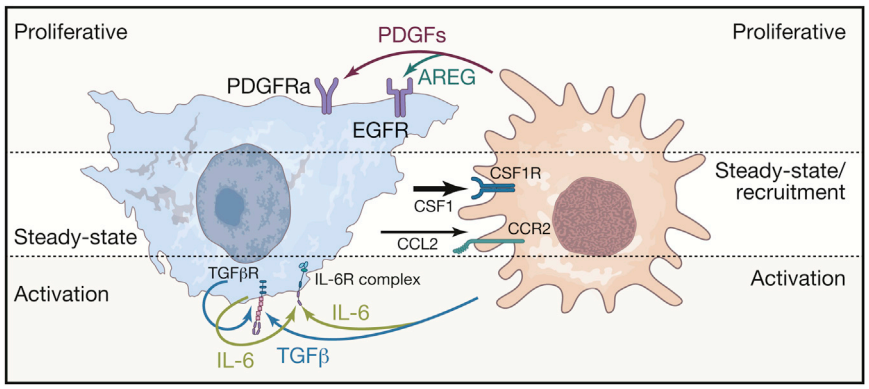

Figure 1. Fibroblast-macrophage interactions during fibrosis
Fibroblasts (left) can provide growth factors, such as CSF1, or chemokines, such as CCL2, to elicit macrophage (right) survival in or migration to fibrotic lesions. Fibroblasts may also secrete TGFβ and IL-6 in an autocrine loop to elicit activation. Activated macrophages secrete PDGF isoforms, TGFβ1, AREG, and IL-6 that can act to elicit proliferation or activate fibroblasts. Fibroblasts produce ECM, which may provide macrophage survival cues (data not shown).

Another axis to highlight with respect to the role of TGFβ in fibroblast-macrophage interactions in fibrosis is amphiregulin (AREG)-epidermal growth factor receptor (EGFR) signaling. AREG promotes fibroblast migration and collagen synthesis in the heart and liver (Liu et al., 2018; Perugorria et al., 2008). This cytokine can be produced by fibroblasts (Poole et al., 2020; Zhou et al., 2012) and activated macrophages (Ding et al., 2016; Minutti et al., 2019). AREG-deficient mice are protected from bleomycin-induced lung fibrosis, and endotracheal transfer of $AREG^{+/+}$, but not $AREG^{-/-}$, activated macrophages exacerbated fibrosis as measured by lung injury (Ding et al., 2016). In a TGFβ1-driven genetic model of lung fibrosis, silencing of AREG via siRNA or EGFR through pharmacologic inhibition significantly diminished fibrosis (Zhou et al., 2012). Zaiss and colleagues showed that *Nippostrongylus brasiliensis* infection, which elicits fibrosis, drives AREG production by alveolar macrophages, promoting the development of activated fibroblasts in the lung in an AREG-dependent manner. Interestingly, using the pericyte-specific $Pdgfrb^{cre}$ system, they showed that AREG promotes activation of latent TGFβ through expression of integrin alpha V to elicit activated fibroblast development (Minutti et al., 2019). It is tempting to speculate that AREG signaling can enable fibroblasts to activate TGFβ, but these studies need to be performed using fibroblast-specific genetic tools.

Overall, the data derived from the fibrosis literature suggest that fibroblasts may engage with macrophages through expression of CSF1 and secretion of macrophage-attractants like CCL2. Fibroblasts may also aid in macrophage activation through provision of IL-6. In turn, activated macrophages can produce factors that drive fibroblast proliferation, like PDGFs and AREG. Macrophages can also promote fibroblast activation through TGFβ, which alone potently drives a fibrotic program, but can also synergize with IL-6, IL-17, and AREG to drive fibroblast activation (Figure 1). IL-1β (Gasse et al., 2007; Wilson et al., 2010) and fibroblast growth factor signaling are implicated in fibrosis (Guzy et al., 2017), but studies have yet to clearly describe a discrete macrophage-fibroblast relationship in these pathways. Additionally, emerging literature suggests that ECM deposited by fibroblasts can provide a survival signal to macrophages, providing another means by which fibroblasts and macrophages may interact during fibrosis (unpublished data). Of note, while macrophages participate in the promotion of fibrosis by activating fibroblasts or driving their proliferation, these cells are also essential for fibrosis resolution (Duffield et al., 2005). Indeed, activated macrophages can degrade excessive ECM deposition via extracellular proteolysis (reviewed in Hinz and Lagares, 2020; McKleroy et al., 2013) or secrete MFGE8 to target collagen for internal uptake and degradation (Atabai et al., 2009; Table 1). Thus, the fibroblast-macrophage circuit can play out in a multitude of ways depending on the nature of the injury, inflammation, and repair. As such, fibrosis severity and duration are likely to be determined by the balance of fibrogenic and resolution activities mediated by paracrine interactions of fibroblasts and macrophages.

FIBROBLAST-MACROPHAGE INTERACTIONS IN THE TUMOR MICROENVIRONMENT

The TME plays a fundamental role in tumor growth and metastasis (reviewed in Binnewies et al., 2018; Winkler et al., 2020). It is composed of various cell types, but fibroblasts and macrophages are recognized as key cellular components of this niche. Accordingly, the mutual interactions between fibroblasts and macrophages play critical roles in many aspects of tumor development (Allaoui et al., 2016; Ireland and Mielgo, 2018; Ronca et al., 2018; Zhang et al., 2019).

Fibroblasts are found in tumors at all stages and referred to here as cancer-associated fibroblasts (CAFs). CAFs are heterogeneous and primarily derived from activated resident fibroblasts (Arina et al., 2016). Multiple subsets of CAFs have been identified in mice and humans, in the same cancer, or across different cancer types (Costa et al., 2018; Dominguez et al., 2020; Elyada et al., 2019; Lambrechts et al., 2018; Pelon et al., 2020; Puram et al., 2017; Tirosh et al., 2016; Vickman et al., 2020; Wu et al., 2020). Various factors including anatomic location and proximity to tumor, cancer type and mutational status, inflammation, disease stage, age, host genetics, treatment history, and experimental procedure should be taken into consideration when comparing or annotating CAF subsets. Currently, there is no clear consensus on the precise role of CAFs in tumor progression. A primary function of CAFs is to synthesize, deposit, and remodel ECM. However, CAFs can also secrete cytokines, chemokines, growth factors, and angiogenic factors (reviewed in Sahai et al., 2020). CAFs are also functionally plastic. For example, serum amyloid A3 (SAA3) is a key mediator of the pro-tumorigenic activity of $PDGFR\alpha^{+}$ CAFs. In an orthotopic pancreatic ductal adenocarcinoma (PDAC) model, genetic ablation of *Saa3* diverted the function of these CAFs from pro- into anti-tumorigenic (Djurec et al., 2018).

Macrophages in the TME are generally referred to as tumor-associated macrophages (TAMs). TAMs can be pro- or anti-tumorigenic for reasons including ontogeny, location, or diversified functional subsets within the TME. Multiple factors contribute to this phenotypic and functional heterogeneity. First, TAMs can be derived from either recruited monocytes or resident macrophages (Cassetta and Pollard, 2018; Engblom et al., 2016). Second, tumor-associated signals can regulate TAM localization and function. As an example, hypoxia induces increased expression of semaphorin 3A (Sema3A) in tumor cells, which acts as an attractant for the migration of TAMs to the hypoxic areas to exert proangiogenic and immunosuppressive functions (Casazza et al., 2013). Here, we focus on how the interactions between fibroblasts and macrophages impact TME and cancer metastasis.

As in the steady state and fibrosis, CAFs and TAMs can interact via the CSF1-CSF1R axis in the TME. For example, CSF1 expression positively correlates with the abundance of $CSF1R^+$ $CD163^+$ macrophages in skin cancer patients, in line with a role of CSF1 in mediating macrophage survival (Neubert et al., 2018). Zhang and colleagues investigated the heterogeneity of TAMs in human cancer patients (Zhang et al., 2020). Using scRNA-seq, they identified two types of TAMs: pro-inflammatory, anti-tumorigenic *C1QC*$^+$ TAMs and more pro-angiogenic, pro-tumorigenic *SPP1*$^+$ TAMs. Analysis of receptor-ligand interactions suggested that *SPP1*$^+$ TAMs were more likely to interact with CAFs via the expression of syndecan-2 on TAMs, which can bind to matrix metallopeptidase 2 (MMP2) on CAFs. The authors went on to show that CSF1R blockade preferentially eliminated *C1QC*$^+$ TAMs and observed that an increased proportion of *SPP1*$^+$ TAMs relative to *C1QC*$^+$ TAMs predicted diminished survival in patients. In a subcutaneous or orthotopic mouse model of hepatocellular carcinoma (HCC), *SPP1*^{hi} tumors had a better response to a combinational therapy of PD-L1 blockade and CSF1R inhibition (Zhu et al., 2019). One possible explanation is that in the *SPP1*^{hi} mice, relatively higher proportion of immunosuppressive TAMs (expressing *SPP1*) were depleted by the CSF1R inhibitors, resulting in a higher degree of the remodulation of the TME favoring tumor suppression.

In addition to fibroblasts, CSF1 can be secreted by tumor cells, suggesting that it may play a pro-tumorigenic role. Consistent with this, in metastatic PDAC, tumor-cell-derived CSF1 induces macrophages to produce granulin, a secreted glycoprotein that promotes fibroblast activation and spurs tumor growth (Elkabets et al., 2011; Quaranta et al., 2018). In addition, Nielsen et al. showed that granulin can drive liver fibroblasts to differentiate into myofibroblasts, thus further amplifying a fibrotic TME (Nielsen et al., 2016). Therefore, in various types of tumors, granulin, which can be expressed in macrophages in response to CSF1, represents a functional mediator linking macrophages and fibroblasts. In another study, Kumar et al., questioned how the response to CSF1 was diverted when the CSF1R signaling in macrophages was interrupted (e.g., CSF1R blockade). They addressed this question in multiple mouse models for lung cancer, melanoma, and prostate cancer. Interestingly, they found that when $CSF1R^+$ TAMs were depleted, CSF1 became more accessible to CAFs, which can also express CSF1R, although at a lower level compared to TAMs. CSF1 promoted myeloid-derived suppressor cell (MDSC) migration to the tumor through CAF-derived CXCL1. Based on these findings, the authors tested the combination of CSF1R blockade and anti-CXCR2 (receptor for CXCL1) and found improved anti-tumor efficacy (Kumar et al., 2017). Together, these studies add more dimensions to the complexity of CSF1-CSF1R signaling in the TME and caution against assuming that fibroblasts and TAMs, respectively, are the only cells producing and consuming CSF1. Indeed, possibly due to this complexity, CSF1R blockade has provided limited therapeutic benefit as a monotherapy in human cancers (Cannarile et al., 2017; Papadopoulos et al., 2017; Ries et al., 2014).

Molecular mechanisms in addition to CSF1-CSF1R signaling can mediate crosstalk between fibroblasts and macrophages in the TME (Table 1). As fibroblasts in fibrosis, CAFs act to drive monocyte recruitment and differentiation. In a mouse model for intrahepatic cholangiocarcinoma (iCCA), Yang et al. reported that FAP^+ CAFs were a major source of CCL2, recruiting circulating $CCR2^+$ myeloid cells, which subsequently gave rise to MDSCs in the TME. Similarly, in human iCCA patients, elevated expression of FAP and CCL2 in stromal compartments correlated with worsened survival (Yang et al., 2016). The CCL2-CCR2 axis linking CAFs and TAMs in promoting tumorigenic stroma formation was also implicated in mouse models of spontaneous lymphoma (Ren et al., 2012). In this model, CAFs produced CCL2, which recruited $CCR2^+$ monocytes and macrophages to the tumors. Genetic ablation of *Ccr2* interrupted the CCL2-CCR2 axis and inhibited tumor growth. This tumor-promoting effect of stroma-derived CCL2 via recruiting immunosuppressive myeloid cells was also found in B16 melanoma and the 4T1 mammary carcinoma (Ren et al., 2012), suggesting a generalized mechanism by which fibroblasts and myeloid cells cooperate to promote tumor growth. In addition to CCR2-dependent macrophage migration, CAFs are the primary source of CXCL14 (Augsten et al., 2009). CXCL14 can amplify a pro-tumor microenvironment, at least in the PDAC models, via stimulating monocyte migration and infiltration and expanding fibroblasts (Augsten et al., 2009). A subset of immunosuppressive $CD163^+$ macrophages elicit the expression of another CXC chemokine, CXCL16, in breast cancer CAFs, which expands fibroblasts in an autocrine manner and recruited more myeloid cells (Allaoui et al., 2016).

CAF can also promote myeloid cell recruitment to the TME independent of CC and CXC chemokines. Davidson et al. examined stromal compartments in murine melanoma and draining lymph nodes (LNs) across different stages (Davidson et al., 2020). They discovered several distinct stromal populations that were conserved between mouse and human tumors, including a subset of $CD34^+$ $PDPN^+$ $PDGFR\alpha^+$ CAFs. These $CD34^+$ CAFs are the main source of complement C3 in melanoma, as well as head and neck cancers (Puram et al., 2017). Increased production of C3 by $CD34^+$ CAFs was found in multiple mouse models and human datasets. C3a, one of the active products of C3, recruited $C3aR^+$ circulating monocytes to the TME. Confocal analysis showed that $C3aR^+$ macrophages located proximally to $CD34^+$ CAFs. Importantly, inhibition of C3a or C3aR diminished the infiltration of $C3aR^+$ macrophages and suppressed tumor growth. Together, these data suggest a conserved CAF-TAM crosstalk through the C3a-C3aR axis in multiple tumors (Davidson et al., 2020).

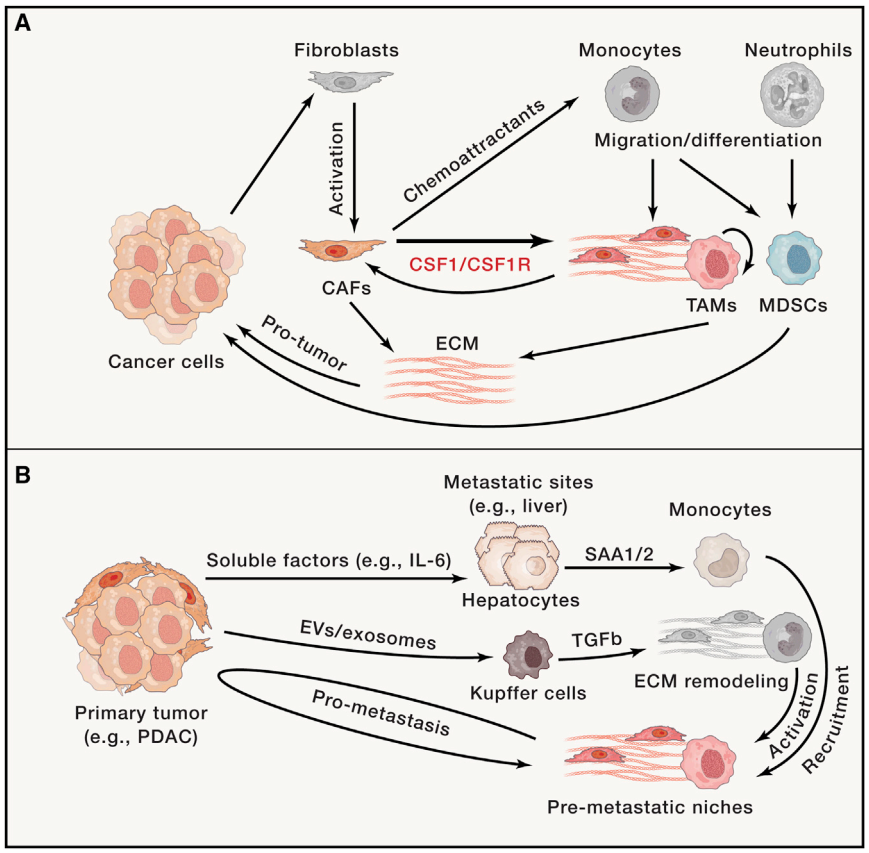

Figure 2. Fibroblast-macrophage interactions in cancer

(A) During the initiation and progression of cancer, resident fibroblasts become activated (by cancer cells in many cases). Activated fibroblasts (termed CAFs) secrete chemokines to attract circulating monocytes or neutrophils to the tumor sites. These cells differentiate into TAMs or MDSCs. Both CAFs and TAMs can remodel tumor ECM by depositing collagens and promoting fibrosis. Together, a pro-tumor microenvironment is formed by specialized subsets of CAFs, TAMs, and MSDCs. Enhanced tumor growth may further promote fibroblast activation, thus forming a loop of interactions and amplifying oncogenesis.

(B) During cancer metastasis, multiple molecular mechanisms have been found to bridge the communications between primary and metastatic sites, with a few of them highlighted here. These studies support a central model whereby soluble factors produced by primary tumors (or the stromal therein) can disseminate to distal organs, where they target various cell types (e.g., hepatocytes and Kupffer cells in the case of liver metastasis) to promote the formation of "pre-metastatic niches," which is characterized by the activation of local fibroblasts and macrophages, and the recruitment of monocytes.

TAMs at primary tumor sites can also instruct local fibroblasts to create an environment that promotes metastatic seeding and growth. Using genetically engineered mouse models of spontaneous breast cancer, Weaver and colleagues found that TAMs (expressing CD163 and RELMα) promoted collagen crosslinking and stromal stiffening. These macrophages secreted TGFβ, which induced lysyl oxidase (LOX) expression in CAFs (Maller et al., 2021). LOX is an ECM-remodeling enzyme that mediates collagen fibrillogenesis and crosslinking (Chen et al., 2015; Levental et al., 2009; Pickup et al., 2013) and promotes lung metastasis of breast cancer (Salvador et al., 2017). In this regard, inhibition of LOX abrogated tumor metastasis (Miller et al., 2015; Salvador et al., 2017). Melanoma cells can stimulate newly recruited monocyte-derived macrophages to secrete IL-1β, which targets CAFs to produce IL-8, a ligand attracting $CXCR2^+$ cells, primarily neutrophils. This positive feedback loop, formed by CAFs and macrophages, rendered melanoma cells resistant to treatment of BRAF and MEK inhibitions. Importantly, genetic ablation of *Il1r* or CXCR2 inhibition synergized effectively with MEK inhibitor treatment (Young et al., 2017).

As in fibrotic indications, TAMs can communicate with CAFs via the provision of PDGFs. Martinet and colleagues found that TAMs that were present in the tumor stroma of human lung tumors express *PDGFA* and *PDGFB* in 59 of the 64 (92%) patients examined by *in situ* hybridization. Moreover, they observed that CAFs expressed *PDGFRA* (Vignaud et al., 1994). *PDGFA* is increased in prostate tumors as compared to adjacent tissues, although it remains unclear whether this expression is exclusive to macrophages (Jones et al., 2015). Indeed, PDGF and PDGFR expression are associated with poor prognosis across many tumor types (Heldin, 2013).

Thus, a general view forms of fibroblast-macrophage crosstalk in tumor ECM formation (Figure 2). In many cases, tumor-derived factors may activate resident fibroblasts to become CAFs, which secrete and deposit ECM components. At the same time, CAFs can recruit monocytes and macrophages via chemokines and other chemoattractive mediators, such as C3a, and promote TAM fitness via the CSF1-CSF1R axis. In this context, a notable feature of TAMs is the acquisition of a pro-fibrotic program (e.g., collagen synthesis and deposition). Meanwhile, TAMs can also act upon fibroblasts via factors like TGFβ to promote fibroblast activation or PDGFs to drive fibroblast proliferation. While CAFs are considered the primary players in tumor ECM formation (reviewed in Kalluri, 2016), the CAF-TAM circuit may reinforce and sustain a tumorigenic matrix. Overall, CAFs and TAMs can form two-dimensional communication circuits in the TME, either with CAFs acting as messengers to "educate" TAMs and their precursors or *vice versa* with TAMs being the upstream instructors to activate resident fibroblasts. Emerging literature indicates that fibroblast-macrophage interactions may also operate as key organizing hubs across tissues that can promote tumor metastasis (Lee et al., 2019; Figure 2). As an example, IL-6 produced by fibroblasts in a primary pancreatic tumor has been shown to activate STAT3 signaling in liver hepatocytes. STAT3 activation, in turn, dramatically promoted the production of serum amyloid proteins SAA1/2 by hepatocytes. SAA1/2 can promote myeloid cell accumulation and liver fibrosis. Genetic ablation or blockade of components of the IL-6/STAT3/SAA axis prevented the establishment of a pre-metastatic niche and inhibited liver metastasis (Lee et al., 2019).

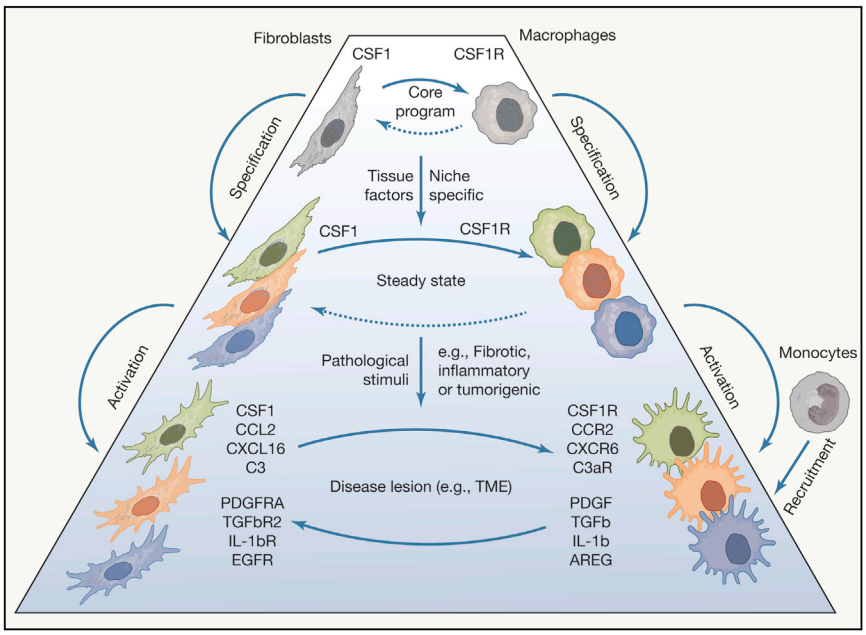

Figure 3. Integrated view of fibroblast-macrophage interactions *in vivo*

Illustrated here is a simplified diagram to recapitulate the key concepts extracted from accumulating studies about fibroblast-macrophage interactions in health, fibrosis, and cancer. First, a core program (mainly via the CSF1-CSF1R axis) serves as a persistent force to connect fibroblasts and macrophages across various conditions. Fibroblasts drive this interaction by producing CSF1 (solid line in the "core program"). Reciprocally, it remains less clear how macrophages initiate the interaction to maintain fibroblasts *in vivo*. Second, in the steady state, tissue-derived factors drive the specification of fibroblasts and macrophages, characterized by the intra-organ and inter-organ heterogeneity of each cell type (depicted by different colors). The CSF1-CSF1R signaling remains robust in this setting. However, whether additional ligand-receptor pairs are induced remains to be identified. Last, upon emergence of pathological stimuli, such as inflammatory or tumorigenic insults, both fibroblasts and macrophages can be activated by signals derived locally or from distal organs. Diverse modes of interactions between fibroblasts and macrophages can be observed, and a few examples of the ligand-receptor pairs are shown. In addition, circulating monocytes are recruited to disease lesions and differentiate into functionally specialized macrophages or MDSCs. All of these factors contribute to further increasing the complexities of the broader fibroblast-macrophage network.

Importantly, the overexpression of SAAs by hepatocytes or increased circulating SAAs can also be detected in pancreatic and colorectal cancer patients with liver metastases (Lee et al., 2019). In addition, tumor-cell-derived extracellular vesicles or exosomes can mediate the communication between cells in primary tumors and distal metastatic sites. For example, pancreatic-cancer-derived exosomes can stimulate liver Kupffer cells to produce TGFβ, which, in turn, induced a cascade of responses, including the activation of liver stellate cells, the differentiation of myofibroblasts, and increased secretion of the matrix component fibronectin, all favoring the formation of pre-metastatic niches (Costa-Silva et al., 2015).

These findings raise the question of why different molecular mediators are used by fibroblasts and macrophages for their communication. Is this due to cancer type, tissue location, or the origins and subtypes of fibroblasts and macrophages? Several studies have attempted to address these questions. DeNardo and colleagues have focused on PDAC, which is characterized by extensive fibrosis and myeloid cell accumulation. They surveyed the origins of TAMs in the KPC genetically engineered mouse model, an autochthonous tumor model that recapitulates many pathological features of PDAC in human patients. Whereas monocyte-derived TAMs favored an antigen-presentation role, embryonically derived TAMs exhibited a strong pro-fibrotic program (Zhu et al., 2017). Importantly, the authors identified a subset of CXCR4^{+} TAMs in human PDAC samples that potentially exhibited a similar pro-fibrotic role, reminiscent of the pro-fibrotic function of embryonically derived TAMs in the KPC mouse PDAC model. Conversely, in an orthotopic colorectal cancer model, which is also characterized by rich stroma and extensive myeloid cell infiltration, Afik et al. found that monocyte-derived TAMs were the key players in remodeling pro-tumor ECM (Afik et al., 2016). Overall, parsing the dynamic interactions between macrophages and fibroblasts in cancer will require the implementation of genetic or pharmacologic tools that enable temporal- and lineage-specific modulation of these cells in diverse tumor types, at different stages of tumor progression, and following therapeutic intervention.

AN INTEGRATED VIEW OF FIBROBLAST-MACROPHAGE INTERACTIONS

Our analysis of the literature suggests that in the steady state and during inflammatory conditions like fibrosis and cancer, fibroblasts can support macrophages in a niche via the provision of CSF1, which can promote survival, be a proliferative cue, or signal expression of a macrophage-specific transcriptional program. During inflammation, fibroblasts continue to provide CSF1 as an essential factor for macrophages. It remains unclear as to whether macrophages provide essential signals that regulate fibroblast homeostasis in tissues during the steady state *in vivo*. However, in fibrosis and the TME, macrophages can provide direct signals to fibroblasts to elicit their proliferation, such as PDGFs and AREG, and provide signals such as TGFβ1, IL-1β, IL-6, and granulin, which can directly activate fibroblasts (Table 1). Fibroblasts and macrophages can also engage in crosstalk indirectly via interactions with the ECM, which fibroblasts can deposit and organize, and macrophages can efficiently degrade and modify. A synthesis of this information suggests a model in which a basal transcriptional program exists for fibroblasts and macrophages and then tissue- or niche-specific signals promote further patterning in the steady state that can be augmented or revised again during inflammation to drive full-on activation (Figure 3).

Multiple studies in what may be considered the bulk genomics era indicated that macrophages integrate environment-specific signals to drive expression of defined transcription factors that enable tissue-specific programming, in parallel with the core macrophage transcriptional program (Gautier et al., 2012; Gosselin et al., 2014; Lavin et al., 2014). Within this framework, CSF1 from fibroblasts may promote a macrophage core transcriptional program while other cell types provide tissue-specification signals. For example, *Wt1*$^{+}$ mesothelial cells, though possibly some *Wt1*$^{+}$ fibroblasts, generate retinoic acid to promote GATA6 in peritoneal macrophages (Buechler et al., 2019), and heme from red blood cells drives SpiC in red pulp macrophages (Haldar et al., 2014) (recently reviewed Blériot et al., 2020; Guilliams et al., 2020). It is interesting to speculate whether single-cell technologies will provide a re-interpretation of macrophage heterogeneity across tissues and diseases.

The fibroblast field is rapidly evolving. The number and names of fibroblast transcriptional subtypes varies per tissue and disease indication. Accordingly, neither a clear nomenclature for fibroblasts and their subtypes nor an overarching conceptual framework to understand this lineage has yet been agreed upon by the field. To this end, work from our laboratory has recently used an integrative single-cell approach to understand the organization of this lineage across tissues, diseases, and species, which may be useful in this regard (Buechler et al., 2021). Nonetheless, across pathologies, including fibrosis and cancer, it is likely that distinct molecular subtypes of fibroblasts can promote specific aspects of disease observations in rheumatoid arthritis (Croft et al., 2019), wound healing (Rinkevich et al., 2015), and cancers (Dominguez et al., 2020).

Fibroblasts and macrophages can operate as a two-cell circuit, as proposed (Zhou et al., 2018) *in vivo*, and in this manner are closely related forms, as noted by Carell and Ebeling. Yet, these cell types rarely operate in isolation in the body, as noted in Guilliams et al. (2020). This is true in fibrotic diseases, such as IPF, where it is likely that epithelial cell damage promotes fibroblast dysregulation, which is then perpetuated by macrophage activation (Sakai and Tager, 2013; Wynn and Barron, 2010). In the TME, fibroblasts and macrophages can interact directly or indirectly through exerting effects on other immune cells, notably T cells. Both CAFs and TAMs can impact T cell migration and function in the TME. They can cooperate to confine anti-tumor T cells in the stroma, blocking their infiltration into tumor sites in human lung cancer patients (Peranzoni et al., 2018). A similar T-cell-confining role of CAFs has also been found in pancreatic cancer, in which CD8^{+} T cells are trapped in the stroma by FAP^{+} CAFs via the CXCL12-CXCR4 axis (Feig et al., 2013). Moreover, multiple immunosuppressive mechanisms, such as IL-10 and TGFβ signaling, are often co-opted by both CAFs and TAMs to impair T cell effector function or promote regulatory T cell (Treg) generation (Mariathasan et al., 2018; Ruffell et al., 2014). In these cases, a synergistic effect can be induced by CAFs and TAMs to suppress anti-tumor T cells. Therefore, while we contend that the health of a tissue or a whole organism may be predicated on the fidelity of the interaction between fibroblasts and macrophages, further contextualization with other cells in mind is required for a complete understanding of the physiology of a tissue or pathology of a disease.

CONCLUDING REMARKS

The ultimate goal of understanding fibroblast-macrophage interactions is to leverage this relationship for therapeutic benefit of patients suffering from fibrosis, cancer or other maladies. To achieve this, a clearer understanding of the phenotypic and functional heterogeneity of fibroblasts and macrophages across tissues and disease conditions using genetic, genomic, imaging, and pharmacological tools is needed. Additionally, the development of human organoid cultures that enable study of multicellular interactions and humanized mouse models that faithfully recapitulate immune cell complexity and tissue compartmentalization will be important to facilitate the translation of these discoveries into the clinic. Recently, others have suggested the possibility that manipulating cell-cell circuits may be a preferred approach over antibody-mediated depletion of macrophages (Guilliams and Svedberg, 2021). However, as fibroblasts may exert direct control over macrophage localization in addition to promoting disease pathogenesis, it may be worth evaluating strategies to directly deplete or reprogram these cells in fibrosis, cancer, and other disease landscapes. Other strategies may employ chimeric-antigen receptor technology on macrophages (Klichinsky et al., 2020) to target CAF or activated fibroblasts in fibrosis, as has been done with CAR-T cells in fibrosis (Aghajanian et al., 2019).

The works and progress we note here have built upon the efforts of Carell and Ebeling and others to better understand how fibroblasts and macrophages work in tandem to achieve homeostasis or to drive pathology. The last 100 years have provided unprecedented clarity on these cell types, and the power of single-cell technologies has yielded essential findings just within the last 5 years. Moving forward, researchers must proceed courageously and validate these transcriptomic observations with orthogonal approaches. While Carell and Ebeling were not always correct (Witkowski, 1980), we agree with their assertion that while the "individuality of the fibroblast and macrophage had become clearly defined…a description of a given structure remains almost without significance if the relations that correlate form and function remain unknown" (Carrel and Ebeling, 1928b). We look forward to the next century of research into fibroblasts and macrophages and believe the field is well on its way to understanding the form and function of these cell types in health and disease.

DECLARATION OF INTERESTS

All of the authors on the manuscript are employees of Genentech. W.F. and S.J.T. are stockholders of Genentech/Roche.

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